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The second species of the endemic Malagasy earthworm genus *Howascolex* Michaelsen, 1901; *Howascolex farafangana* sp. n. (Clitellata, Megadrili)

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Abstract

A recent survey of earthworms carried out in South East Madagascar resulted in collecting two species belonging to the endemic Malagasy earthworm genus *Howascolex* Michaelsen, 1901. One of the species proved to be identical to the generotype *Howascolex madagascariensis* Michaelsen, 1901. The other species *Howascolex farafangana* **sp. n.** represents the second species of the genus. The genus *Howascolex* was originally classified in the subfamily Octochaetinae Michaelsen, 1900. However, its close morphological similarity with the Malagasy acanthodriline species implies a homoplasious origin of the meroic excretory system of *Howascolex* and its inclusion in the family Acanthodrilidae Claus, 1880.

Keywords

Oligochaeta, Octochaetidae, Acanthodrilidae, holonephridia, meronephridia, phylogeny

Introduction

The genus *Howascolex* was described by Michaelsen (1901) for his peculiar species *H. madagascariensis* Michaelsen, 1901 found in a cave near Andrahomana, South Madagascar. This new species possessed a very characteristic excretory system composed of

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praeclitellar holonephridia accompanied by 4–6 meronephridia from the middle of the body. Because of the presence of meronephridia, the new genus was placed into the meroic subfamily Octochaetinae Michaelsen, 1900. Another characteristic feature of *H. madagascariensis* was the large intramural calciferous glands in 16.

Michaelsen (1922) added 3 more species to the genus; *bidens*, *corethrurus*, and *merkaraensis* found in Southern India. Although, these new species shared the characteristic intramural calciferous glands in 16 with *H. madagascariensis* they differed from the Malagasy type in the structure of the nephridial system being purely meroic with caudal megameronephridia found in many other Indian octochaetids (e.g. *Eutyphoeus* Michaelsen, 1900, *Eudichogaster* Michaelsen, 1902).

The genus *Howascolex* was revised and redefined by Pickford (1937) to include several meroic species from Central and South America like *Wegeneriella beauforti* Michaelsen, 1933, *Eodrilus irpex* Michelsen, 1911, *Eodrilus eiseni* Michaelsen, 1911 and *Wegeneriella michaelseni* Černosvitov, 1934. Černosvitov (1939) did not agree with moving the South American *Wegeneriella michaelseni* and *Wegeneriella beauforti* into the Malagasy genus *Howascolex* and on the basis of presence of extramural calciferous sacs in 14–15 proposed a new genus *Wegeneriona* to accommodate them.

Graff (1957) describing several Salvadorian meroic earthworm species erected a new subgenus *Howascolex* (*Graceevelynia*) for the Neotropical species differing from the nominal subgenus by the more anterior calciferous glands (in the region of 8–13 instead of 16). This placement was later refuted by Gates (1962) because all the Central and South American species relegated previously to *Howascolex* by Pickford (1937) and (Graff 1957) are purely meroic and lack holoic body part. Therefore, Gates (1962) moved all these species to *Ramiellona* Michaelsen, 1935.

Julka (1988) revising the octochaetid earthworm fauna of India recognized, that the three *Howascolex* species described by Michaelsen (1922) from India possess enteroic nephridial system, unlike the type species *Howascolex madagascariensis* , which is exoic. Therefore he erected for these, and also several newly described species from India, a separate genus *Wahoscolex* Julka, 1988 leaving *Howascolex* from Madagascar monotypic.

In 2015 the second and third authors conducted earthworm collections in the Farafangana region, South Eastern Madagascar resulted in collecting two species attributable to the genus *Howascolex*. One species proved to be identical with *H. madagascariensis* Michaelsen, 1901, the other represents a species new to science and hitherto described.

Material and methods

Study area. The new collecting sites are situated at the Farafangana district, Vatovavy Fito Vinany region, South East Madagascar (Fig. 1).

Methods. Earthworms primarily were collected by using the diluted formaldehyde method (Raw 1959) supplemented by digging and hand-sorting. The material col-

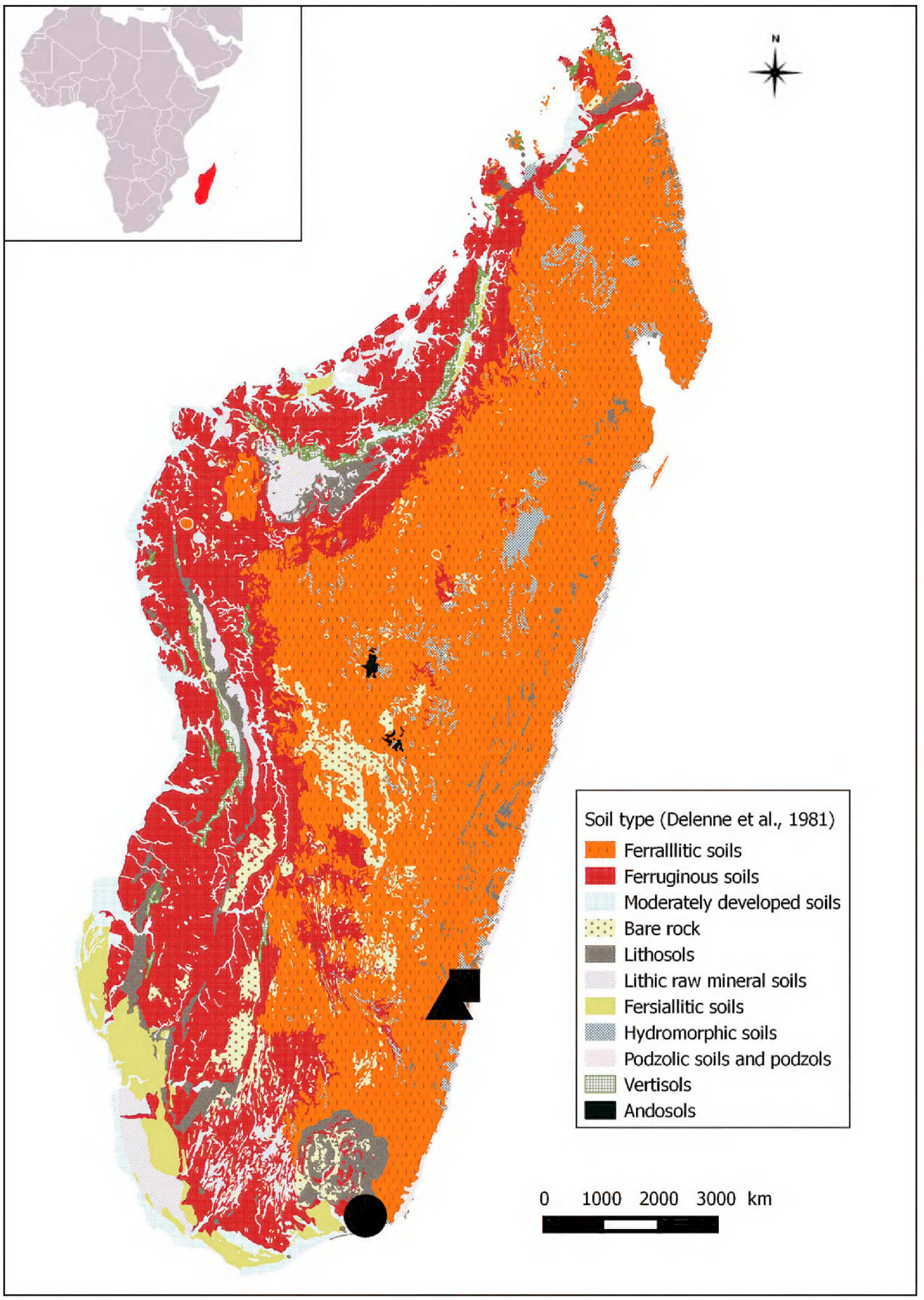


Figure 1. Sampling localities in Madagascar. Black dot = *H. madagascariensis* type locality, black square = *H. madagascariensis* new record, black triangle = *H. farafangana* sp. n.

lected was killed in 75% ethanol and fixed in 4% formaldehyde solution. From each morpho-species parallel material was conserved in 96% ethanol for DNA studies.

Deposition. The material collected is deposited in the Hungarian Natural History Museum (HNHM) and in the National Institute of Biological Resources (NIBR).

Taxonomy

Family Acanthodrilidae Claus, 1880
Genus *Howascolex* Michaelsen, 1901

Howascolex madagascariensis Michaelsen, 1901

Howascolex madagascariensis Michaelsen, 1901: 202, Pickford 1937: 605, Julka 1988: 344.

Diagnosis. Length 90–100 mm diameter 4–6 mm. Colour pale, pigmentation lacking. First dorsal pore in 12/13. Clitellum 13–19. ♀ 14 paired, presetal in *a–a*. Prostatic pores 17, 19. Spermathecal pores on the rim of 8 and 9, spermathecae with elongated oval ampoule, and a small duct bearing an acinous diverticula almost encircling the duct. Gizzard large in 5 or 6, last pair of hearts in 13. Calciferous glands large intramural encircles the oesophagus in 16. Excretory system holoic? before clitellum and meroic with 5–6 meronephridia and a stomate ventralmedian megameronephridium per side. Penial setae present. Ectal third highly bent, tip somewhat spoon-shaped. Length 2 mm diameter 0.04 mm, ornamentation small scattered teeth.

Material examined. ZMUH V5616 *Howascolex madagascariensis* syntypes 8 ex. S. Madagaskar, Andrahomana in Höhlen. Leg. Sikora.

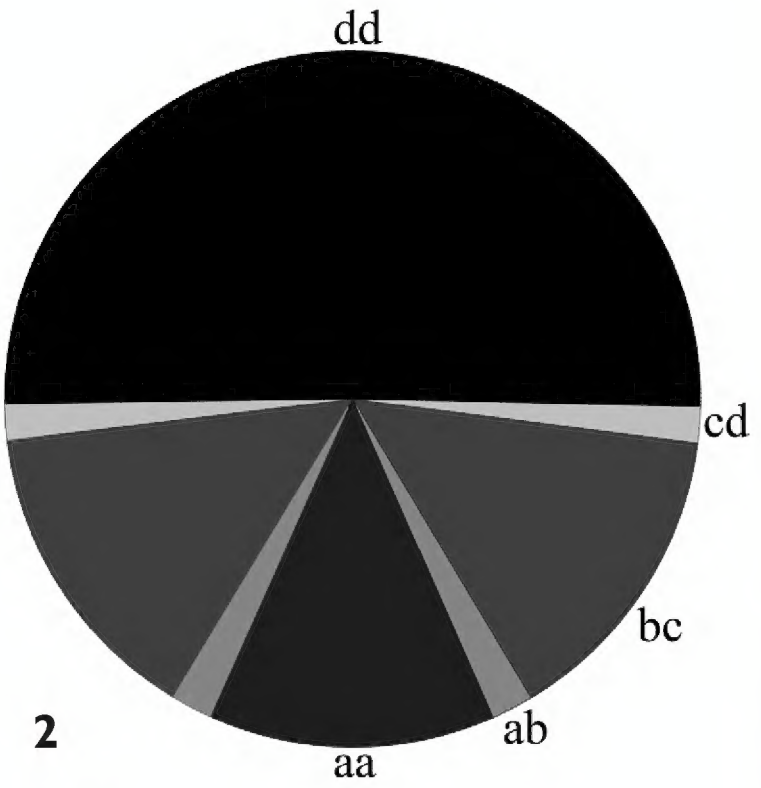
HNHM/AF5667 1+1 juvenile ex., HNHM/AF5668 1 ex. Madagascar, Vatovavy Fito Vinany Region, Farafangana District, Tsararafa, Ankazomafaitsa 22°38'42"S, 47°50'04"E, 63 asl, Legit: Y. Hong & M. Razafindrakoto.

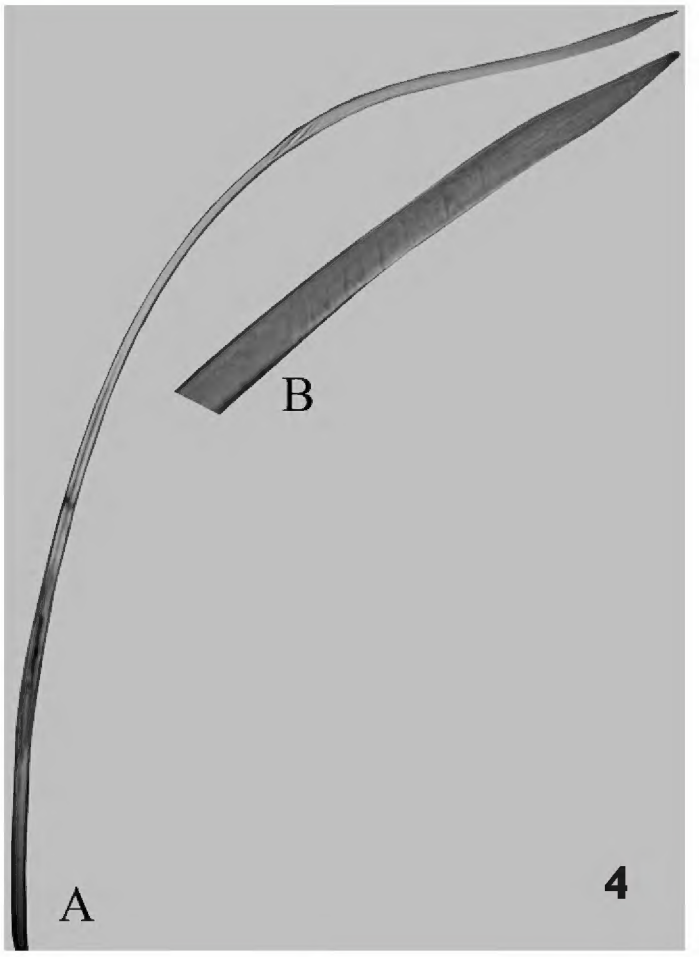
Remarks. We had the possibility to examine the syntypes of *Howascolex madagascariensis* housed in the Zoological Museum of the University of Hamburg (ZMUH). The specimens are softened but all the inner structures are clearly seen. The nephridial system is completely identical with that of described in Michaelsen (1901). In the praeclitellar segments there is a pair of highly coiled stomate, exoic and avesiculate tubular holonephridium. In the postclitellar region the larger nephridia (megameronephridia) are accompanied by several smaller highly coiled meronephridia.

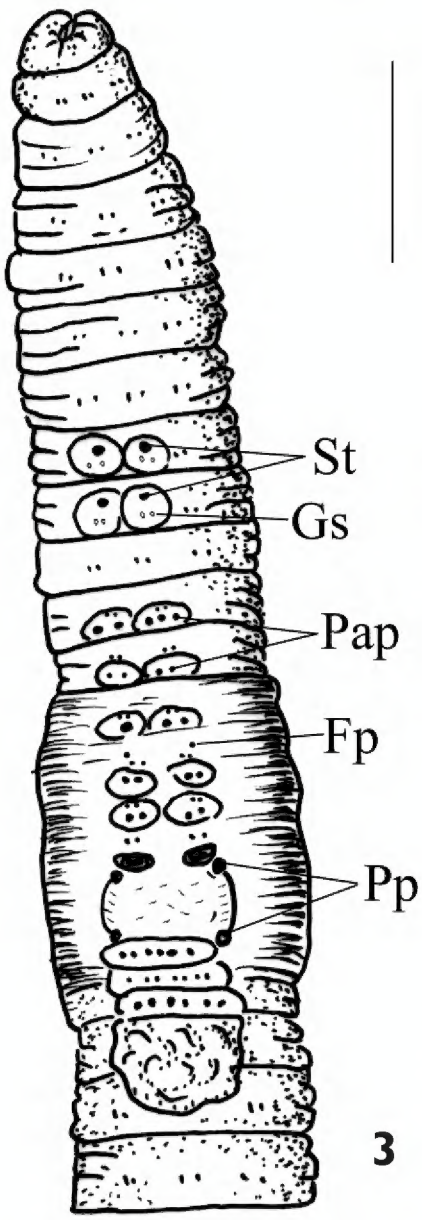
Our specimens agree well with the syntypes but their nephridia are not so well preserved and it seems that the praeclitellar holonephridium consists of 2–3 interconnected more compact regions which became separated after the clitellum to form 4–6 normal meronephridia. The shape of the spermathecae, the penial setae, and the distribution of the characteristic genital papillae are identical in the syntypes and the new material.

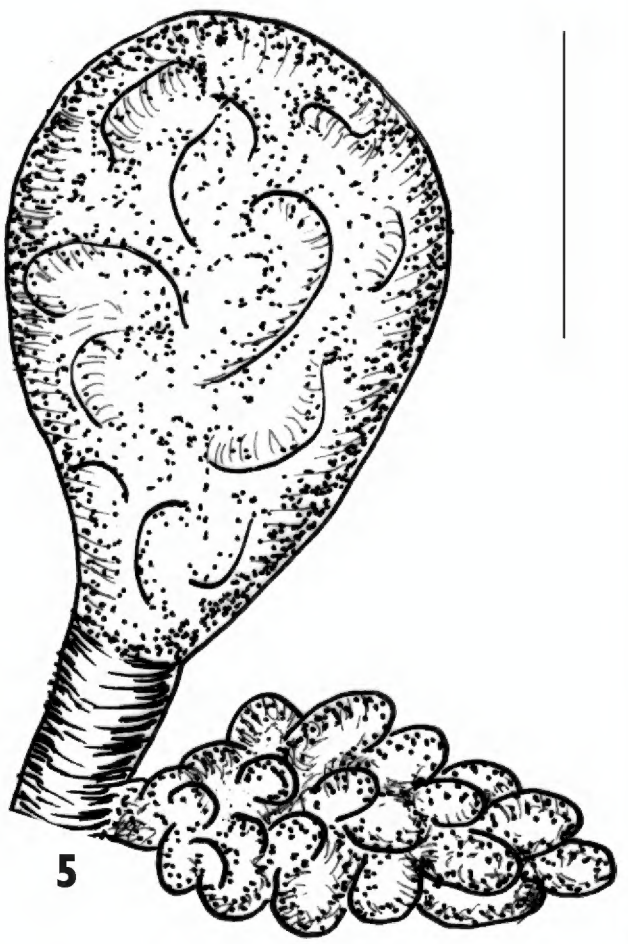
Howascolex farafangana sp. n.
http://zoobank.org/EF428CBA-D5EA-4170-AE2F-9089BDC10350
Figs 2–6

Etymology. Noon in apposition, refers to the type locality; Farafangana district, SE Madagascar.









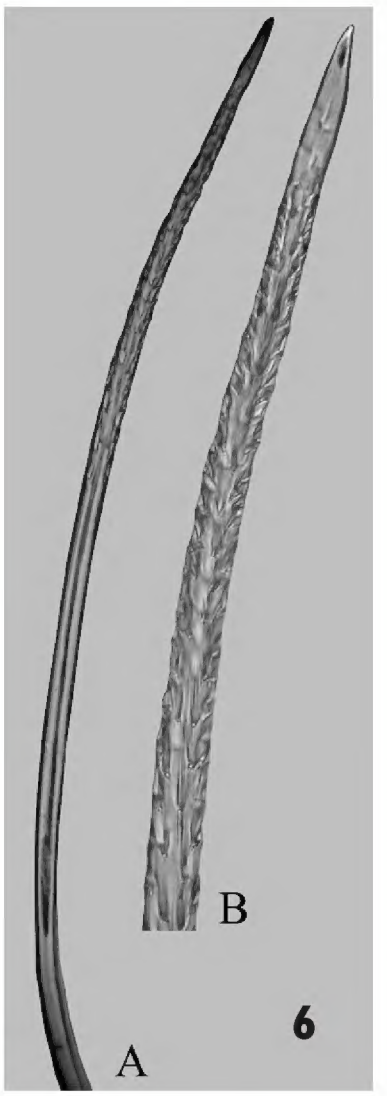


Figure 2–6. *Howascolex farafangana* sp. n. **2** Setal arrangements; *a*, *b*, *c*, *d* represent setal lines **3** ventral view of the fore-body; *Fp* = female pore, *Gs* = genital setal pore, *Pap* = genital papillae, *Pp* = prostatic pores, *St* = spermathecal pore*s* (scale bar = 5 mm) **4** Penial seta, *A* = the whole seta, *B* = tip if the penial seta **5** Spermatheca (scale bar = 0.5 mm) **6** Genital seta, *A* = the whole seta, *B* = tip of the genital seta.

Description. ***External***: Length of the Holotype 155 mm, width (postclitellar) 7 mm. Segments No. 205. Paratypes 142–150 mm in length and 6–7 mm in diameter, segment No. 186–228. Color greyish, pigmentation absent. Prostomium schizolobous, dorsal pores from 9/10. Segments from 3–12 bi- or triannulate, after clitellum with three or four ringlets. Setae visible from segment 2, eight per segment in closely paired regular rows. Setal formula after clitellum aa:ab:bc:cd:dd = 9.2:1.4:10:1:40 (Fig. 2). Setae *a* and *b* of 8, 9 modified to genital setae, of 17, 19 modified to penial setae. Spermathecal pores paired, presetal small slits on segment 8, 9 between *a–b*. Clitellum saddle-shaped on 13–19. Female pores in 14, presetal and slightly lateral to *b*. Two pairs of prostatic pores in 17 and 19 just at the base of seta *b*, joined by slightly convex seminal grooves, running outside *b*. Male pores minute in 18, within the seminal grooves. Genital marks unpaired as 2–3 mid-ventral, intra and intersegmental papillar lines located in the region 19–22 and paired group of small postsetal papillae on 8, 9, 10, 12 in setal lines *a–b* (Fig. 3)

Internal: First septum visible 6/7, septa 8/9, 12/13–13/14 thickened, 9/10–11/12 strongly muscular. One large oesophageal gizzard in 5 or 6, extending up to segment 7. Dorsal vessel single throughout, last pair of hearts in 13. Excretory system with 2–3 coiled (interconnected?) exoic and stomate nephridial tufts per side before clitellum. After clitellum their number increases to 5–6 discrete biramous meronephridia and a ventral megameronephridium per side. Large intramural calciferous gland present in segment 16 sometimes bulging into segment 17 as well. Intestine begins in 20 bearing medium-sized lamellar typhlosole from 23. Lateral typhlosoles lacking.

Holandric. Two pairs of testes in 10, 11. Male funnels iridescent and plicated, testis sacs missing. Two pairs of seminal vesicles in 11 and 12, attached respectively to septa 10/11 and 11/12. One pair of moderate size ovaries in 13. Two pairs of tubular prostates of similar size in 17 and 19, highly coiled and confined to their own segment. Penial setae 3.5 mm in length and 0.025 mm in diameter. The ectal third bent, tip slightly lanceolate, ornamentation fine transversal serrations (Fig. 4).

Two pairs of spermathecae opening segmentally on 8 and 9. Ampulla slightly elongate, oval, duct short. Diverticulum front-facing, acinous, joins to the duct just above the spermathecal pore (Fig. 5). Genital setae almost straight, 1.8 mm long, and 0.035 mm in diameter. Tip lanceolate, ornamentation dense sharp scales (Fig. 6).

Holotype. HNHM/AF5664. Madagascar, Vatovavy Fito Vinany, Farafangana, Tsararafa, Anrazomafaitsa. 22°43'35"S, 47°47'38"E, 28 m asl. Legit: Y. Hong & M. Razafindrakoto 20. 02. 2015.

Paratypes. HNHM/AF5665, 3 ex. Locality and date same as that of the Holotype. HNHM AF/5666, 2 ex. Madagascar, Vatovavy Fito Vinany, Farafangana, Tsararafa, Anrazomafaitsa 22°38'42"S, 047°50'4"E, 63 m asl. Legit: Y. Hong & M. Razafindrakoto 18. 02. 2015.

Remarks. This second species of *Howascolex* differs from the generotype by its larger size, the segmental spermathecal pores and in the presence of genital setae. The presence of a moderately sized typhlosole is also a difference, however the original description is erroneous reporting complete lack of typhlosole in *H. madagascariensis*.

Opening the intestine just after the prostatic segments a small twine-like typhlosole can be observed running along the dorsal surface of the intestine.

Disscussion

The genus *Howascolex* was originally classified in the subfamily Octochaetinae within the highly heterogeneous family Megascolecidae, consisted also of Acanthodrilinae, Ocnerodrilinae and even Eudrilinae as subfamilies (Michaelsen 1900). The treatment of the subfamily Octochaetinae was quite heterogeneous in the subsequent revisions of the earthworms' family system (e.g. Stephenson 1930, Lee 1959, Gates 1959, Jamieson 1971) until Sims (1980), mainly following Gates (1959), advocated a "pragmatic" solution elevating the previous subfamilies of the catch-all family Megascolecidae to family rank. In this system Octochaetidae was defined by the presence of tubular prostates in acanthodriline arrangement (basically two pairs of prostates in 17, 19, male pore in 18) and a meroic excretory system, while Acanthodrilidae differed from it only by its holoic nephridia.

This classical system was severely criticized by Csuzdi (1996, 2010) demonstrating that the meroic excretory system is highly homoplasious and therefore the family Octochaetidae so defined is polyphyletic. Although this view was not generally supported (e.g. Blakemore 2005, 2013) the accumulating molecular data suggest that the classical Octochaetidae is highly polyphyletic and the meroic states of the nephridial system were acquired independently in the different lineages (Buckley et al. 2011, James and Davidson 2012).

From this point of view the position of *Howascolex* is very interesting. Michaelsen (1901) and Stephenson (1930) hypothesized that *Howascolex* is basal to any other octochaetids with its composite (praeclitellar holoic and postclitellar meroic) excretory system. However it was noticed even by Michaelsen (1901) that the octochaetid *Howascolex* shows many similarities with the acanthodrilid species recorded by that time from Madagascar (*Acanthodrilus majungianus* Michaelsen, 1897 and *Acanthodrilus voeltzkowi* Michaelsen, 1897) e.g. the structure of spermathecae, the position of the last pair of hearts and the presence of post ovarial calciferous glands are all shared characters between these three species. Therefore he re-examined the nephridial system of the two acanthodriline species and recognized, that in *voeltzkowi* it is purely holoic, but in *majungianus* it cannot be determined due to the bad conservation.

The nephridial system of both species reported here is quite peculiar, consisting of very small repeatedly coiled tubes in the front of the body and a truly meroic system in the postclitellar segments. However, the similarity in the structure of spermathecae and also the presence of intramural calciferous glands in one or several post-ovarial segments suggest that they are closely related to the Malagasy acanthodriline species and the meroic excretory system evolved independently in the island. Consequently, the *Howascolex* and '*Acanthodrilus*' species of Madagascar seem to form a monophyletic clade advocating also a rejection of the classical Octochaetidae family. To decide whether this scenario is right or *Howascolex* belongs to the Indian clade of octochaetid-like worms a detailed molecular study is needed.

Acknowledgements

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